purification of the separated heparinases by gel permeation HPLC, and

recovering the heparinases I, II, and III separated by gel permeation HPLC.

Remarks

The Amendment mailed March 9, 1994

It is the understanding that the Amendment mailed March 9, 1994, was not entered. The claims as presented herein are amended based on the Amendment mailed November 8, 1993.

The claims have been amended to more clearly define the purified enzyme as expressed in *F. heparinum*, although it could be expressed in other bacterial hosts, if the gene encoding the enzyme is inserted into an appropriate vector. This has been demonstrated by others prior to filing of this application for heparinase I (expressed in *E. coli*); others have subsequently isolated the genes for heparinase II and III and expressed these in non-*F. heparinum* organisms. Regardless of which bacterial system the enzyme is expressed in, however, the enzymes have the same characteristic that are claimed in claims 1 and 2 and can be used to isolate the enzyme, as defined by the method claims.

Rejections under 35 U.S.C. §112

The genus and species of the named bacteria have been italicized, not underlined, as is proper. To underline the name

of the bacteria in a claim would create undue and unnecessary confusion.

The specification and claims have been objected to under §112 on the basis that the organism *F. heparinum* must be readily available to the public. This objection and rejection was previously traversed in view of documents which indicates that the organism can be obtained from the American Type Culture Collection, Rockville, MD, without restriction, upon payment of a fee, as demonstrated by the invoice referencing a page from the ATCC catalogue showing that applicants purchased *F. heparinum* from the ATCC. Additional pages are enclosed from the 1992 ATCC catalogue providing ordering information and current prices.

The Examiner's attention is drawn to the January 4, 1994, Official Gazette, (62) Deposit of Biological Materials for Patent Purposes. Under "Need or Opportunity to Make a Deposit [§1.802], referring to the decision in In re Metcalfe, 410 F.2d 1378, 161 USPQ 879 (CCPA 1969), it is specifically stated that the "examiner should not be unduly concerned about continued access to the public". Availability through the ATCC should certainly constitute availability of reagents under normal circumstances.

Claim 6 has been amended using the language suggested by the Examiner at page 3, last paragraph of the Office Action,

D

to use the term "biologically pure" to describe the bacterial culture, and to incorporate recovery steps.

The claim has not been amended to define how the bacterial cells are lysed since to do so would allow anyone to take advantage of applicants' discovery and avoid the claim. The The statute not only does not require limiting the claim to one embodiment when numerous equivalent methods are known, and have been known for decades, but the Courts have created the doctrine of equivalents to further expand the scope of claims in similar circumstances. 35 U.S.C. §112 requires that the claimed invention be defined with sufficient clarity that one of ordinary skill in the art would know what was encompassed by the claim; even a high school biology student knows of alternative ways to lyse a bacteria!

Rejection under 35 U.S.C. §103

Claims 1-9 have been rejected under 35 U.S.C. §103 as obvious over U.S. Patent No. 5,169,172 to Zimmerman, et al., in combination with U.S. Patent No. 5,198,355 to Kikuchi, et al. These rejections are respectfully traversed.

No where have applicants admitted that the claimed method is taught by the prior art; nor has the Examiner, since the rejection is made under 35 U.S.C. §103.

The language regarding "free of lyase activity other than heparinase II or III" has not been deleted although the

undersigned is willing to discuss language changes which would clarify the intent: the prior art describes heparinase preparations; it does not show purified heparinase II or III which is totally free of other lyases, including heparinase I, II or III, respectively, chondroitinase A or chondroitinase B. The term clearly defines that the purified heparinase does not contain lyase activity derived from an enzyme other than the claimed enzyme; *none of the prior art discloses such a preparation*.

It is important to understand that although it was known that *F. heparinum* had multiple lyase activities, it was not clear that there were three distinct enzymes, at least one of which also had multiple substrate specifities.

The three heparinases are defined as follows:

	heparinase I	heparinase II	heparinase III
names	heparinase EC 4.2.2.7	heparitinase III	heparitinase heparinase heparitinase I EC 4.2.2.8
mw[a]	42,800	84,100	70,800
substrate specificityd[d]	heparin	heparin and heparan sulfate	heparan sulfate
pH optimum[b]	9.1-9.2	8.9-9.1	9.9-10.1
temperature optimum[c]	35°C	40°C	45°C

a. Page 21, lines 25-27.
b. Page 24, lines 9-13.
c. Page 28, lines 16-19.
d. Figure 5; page 29, lines 3-18; Table III, page 30.

Claims 1 and 2 have also been amended to define the claimed heparinases by their molecular weight, substrate specificity, and pH optimum.

Zimmerman, et al.

Zimmerman, et al., discloses that one can isolate two heparinases from *F. heparinum*: heparinase I, the 45,000 mw enzyme, and heparinase II, which is asserted to have a molecular weight of about 70,000. The latter preparation was not a pure enzyme, although it was distinct from heparinase I. Accordingly, it would have been contaminated with at least chondroitinase A and B.

Zimmerman provides no incentive for one to look for a third heparinase having substrate specificity solely for heparan sulfate - the assay was conducted solely using **heparin** as the substrate; there is no disclosure of a second heparinase (i.e., heparinase II) having substrate specificity for both heparin and heparan sulfate with a molecular weight of 84,100.

It cannot be obvious from Zimmerman to look for enzymes cleaving heparan sulfate but not heparin when no assay for heparan sulfate is suggested or used (see col. 3, lines 40-49). Therefore it cannot be obvious from Zimmerman, et al., to isolate heparinase III.

It also cannot be obvious to isolate a heparinase cleaving both heparin and heparan sulfate with a molecular weight of 84,100, when the two enzymes that were isolated had molecular weights substantially less: 42,000 and 70,000 (col. 6, lines 55-57).

Kikuchi, et al.

Kikuchi, et al., merely discloses that one can purify glycosaminoglycans.

The combination of the Art

There is no motivation, alone or in combination with Zimmerman, et al., to isolate three heparinases from *F. heparinum* having the claimed molecular weights, pH optimum, substrate specificity, nor purity and freedom from **other** lyases, based on

either Zimmerman, et al., or Kikuchi, et al. Accordingly, the claimed enzymes and method for purification cannot be obvious from the cited art.

Allowance of claims 1-3, and 5-9, as amended, is respectfully requested.

Respectfully submitted,

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Dated: June 8, 1994

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CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Date: June 8, 1994

Patrea L. Pabst

1. (two times amended) A purified heparinase II [isolated from] present in *Heparinum flavobacterium* free of lyase activity other than heparinase II activity, having a molecular weight of 84,100, cleaving heparin and heparan sulfate and having a pH optimum of 8.9-9.1.

2. (two times amended) A purified heparinase III [isolated from] which is expressed in *Heparinum flavobacterium* free of lyase activity other than heparinase III activity, having a molecular weight of 70,800, cleaving heparan sulfate, and having a pH optimum of 9.9-10.1.

3. The heparinase III of claim 2 which does not cleave heparin sulfate.

Please cancel claim 4.

5. (amended) The heparinase III of claim [4] 2 stabilized with albumin.

6. (twice amended) A method for purifying heparinase I, II, and III from a biologically pure culture of *Heparinum flavobacterium* comprising the steps of

lysing *Flavobacterium heparinum* cells in a biologically pure culture of *Flavobacterium heparinum*,

removing cell debri and nucleic acids from the cell lysate,

absorption of heparinase I, II, and III to hydroxyapatite,

absorption of non-heparinase I, II, and III proteins to QAE-resin,

recovery of the heparinase I, II, and III not bound to the QAE-resin,

separation of heparinase I, II, and III by HPLC on a hydroxylapatite column,

recovery of the heparinase I, II, and III separated on the hydroxylapatite column,

purification of the separated heparinases by cation exchange FPLC, [and]

recovery of the heparinase I, II, and III separated by cation exchange FPLC,

purification of the separated heparinases by gel permeation HPLC, and

recovering the heparinases I, II, and III separated by gel permeation HPLC.

7. The method of claim 6 wherein the nucleic acids are removed by precipitation with protamine.

8. The method of claim 6 wherein the heparinases are separated on the hydroxylapatite column by elution with a salt gradient.

9. The method of claim 6 wherein the heparinases are eluted from the cation exchange column by a gradient of increasing salt concentration.

Please cancel claims 10-17.